

The biopsies may be taken from different sites of a single individual or from a number of individuals.

A virus used in a method as described herein will desirably cause few or only minor clinical 5 symptoms in the recipient. Such viruses are readily obtainable from commercial sources well known to the skilled addressee and can be screened for their effectiveness in the instant methods in the manner described above. Desirably, the virus will normally be an echovirus selected from the group consisting of Echovirus EV1 and Echovirus EV8. Each of these viruses recognise $\alpha_2\beta_1$ for cell infectivity. EV1 has for instance been associated with mild upper respiratory illnesses and 10 also pleurodynia (Fields B. N. et al, 2000; McCracken A. W. et al, 1969).

The expression of $\alpha_2\beta_1$ is believed to be upregulated on ovarian carcinomas due to the prevalent collagen I matrix it encounters in the mesothelial. Numerous malignant melanomas have also been shown to express upregulated levels of $\alpha_2\beta_1$ (Kramer R. H. and Marks N, 1989; Ramos D. M. 15 et al, 1990). EV1 and collagen attach to $\alpha_2\beta_1$ using different residues in domain I of the $\alpha_2\beta_1$ subunit (Bergelson J.H. 1993). The integrin $\alpha_2\beta_1$ cannot simultaneously accommodate EV1 and collagen. However, the virus binds $\alpha_2\beta_1$ with a 10-fold increase in affinity compared to collagen I (Xing L, 2002).

20 For the purpose of screening a given virus to ascertain whether it is capable of infecting and causing the death of malignant cells, malignant cell lines may be used rather than primary malignant cells isolated from a biopsy.

The selected virus will preferably be injected directly into a number of sites on a malignant tumor 25 in order to maximise the area for potential infection of the tumor by the virus. Rather than intact virus, viral or other plasmids or expression vectors incorporating nucleic acid for generation of the virus may be injected into the tumor for uptake by tumor cells and generation of intact virus within the cells for effecting the treatment. Suitable expression vectors include plasmids capable of expression of a DNA (eg genomic DNA or cDNA) insert encoding viral proteins necessary for 30 generation of the virus. An expression vector will typically include transcriptional regulatory control sequences to which the inserted nucleic acid is operably linked. By "operably linked" is meant the nucleic acid insert is linked to the transcriptional regulatory control sequences for permitting transcription of the inserted sequence(s) without a shift in the reading frame of the insert. Such transcriptional regulatory control sequences include promotors

for facilitating binding of RNA polymerase to initiate transcription, and expression control elements for enabling binding of ribosomes to transcribed mRNA.

More particularly, the term "regulatory control sequence" as used herein is to be taken to
5 encompass any DNA that is involved in driving transcription and controlling (ie regulating) the level of transcription of a given DNA sequence. For example, a 5' regulatory control sequence is a DNA sequence located upstream of a coding sequence and which may comprise the promotor and the 5'untranslated leader sequence. A 3' regulatory control sequence is a DNA sequence located downstream of the coding sequence(s), which may comprise suitable transcription termination
10 (and/or) regulation signals, including one or more polyadenylation signals. As used herein, the term "promotor" encompasses any DNA sequence which is recognised and bound (directly or indirectly) by a DNA-dependant RNA polymerase during initiation of transcription. A promotor includes the transcription initiation site, and binding sites for transcription initiation factors and RNA polymerase, and can comprise various other sites or sequences (eg enhancers), to which
15 gene expression regulatory proteins may bind.

Numerous expression vectors suitable for transfection of mammalian cells are known in the art.
Expression vectors suitable for transfection of mammalian cells include pSV2neo, pEF-PGk.
puro, pTk2 and non-replicating adenoviral shuttle vectors incorporating the polyadenylation site
20 and elongation factor 1-x promotor and pAdEasy based expression vectors most preferably incorporating a cytomegalovirus (CMV) promotor (eg see He et al, 1998). The plasmid pEFBOS which employs the polypeptide elongation factor- alpha 2 as the promotor may also be utilised.

cDNA encoding the viral proteins necessary for generation of the virus may be prepared by
25 reverse transcribing the viral RNA genome or fragments thereof and incorporated into a suitable vector utilising recombinant techniques well known in the art as described in for example Sambrook et al (1989), Molecular Cloning: A Laboratory Manual, Second Ed., Cold Spring Harbour Laboratory Press, New York, and Ausubel et al. , (1994), Current Protocols in Molecular Biology, USA, Vol. 1 and 2.

30 Rather than cDNA, cells may be transfected with viral RNA extracted from purified virions or for instance RNA transcripts may be generated invitro from xDNA templates utilising bacteriophage T7 RNA polymerase as described in Ansardi D C., et al, 2001. Similarly, a single plasmid or RNA molecule may be administered for expression of viral proteins and generation of virus, or a plurality of plasmids or RNA molecules encoding

Claims:

1. A method for treatment of abnormal cells in a mammal, the method comprising treating the mammal with an effective amount of virus selected from 5 echoviruses, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$ for infectivity of the cells such that at least some of the cells are killed by the virus.
2. A method according to claim 1 comprising subjecting the mammal to a number of treatments with the virus, and the virus in each of the treatments is the same or different.
- 10 3. A method according to claim 1 wherein the virus comprises an echovirus serotype or modified form thereof.
4. A method according to claim 3 wherein the virus is selected from the group consisting of EV1 and EV8.
5. A method according to claim 3 wherein the virus is a modified echovirus.
- 15 6. A method according to claim 5 wherein the virus has been modified to enhance ability of the virus to infect the abnormal cells.
7. A method according to claim 5 or 6 wherein the modified echovirus is a modified form of an echovirus selected from a group consisting of EV1 and EV8.
- 20 8. A method according to any one of claims 1 to 7 wherein the virus is administered to the mammal in combination with a further virus which infects the abnormal cells.
9. A method according to claim 8 wherein the abnormal cells express ICAM-1 and the further virus recognises ICAM-1 for infectivity of the abnormal cells.
10. A method according to claim 9 wherein the further virus is a Coxsackievirus 25 or modified form thereof.
11. A method according to claim 10 wherein the Coxsackievirus is a Coxsackievirus serotype selected from A13, A15, A18 and A21.
12. A method according to any one of claims 1 to 11 wherein the abnormal cells are cancer cells.
- 30 13. A method according to claim 12 wherein the cancer cells are cells of a cancer selected from a group consisting of ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer and colorectal cancer, or have spread from ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer or colorectal cancer.

14. A method according to any one of claims 1 to 13 wherein the abnormal cells have up-regulated expression $\alpha_2\beta_1$.

15. A method according to any one of claims 1 to 14 wherein the virus is administered topically, systemically or intratumorally to the mammal.

5 16. A method of screening a sample of abnormal cells from a mammal for susceptibility to virus induced cell death to evaluate administering virus to the mammal for treatment of the abnormal cells, the method comprising:

(a) providing the sample of the abnormal cells;

10 (b) treating the cells with the virus for a period of time sufficient to allow infection of the cells by the virus; and

(c) determining whether the virus has infected and caused death of at least some of the abnormal cells;

wherein the virus is selected from echoviruses, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$ for infectivity of the abnormal cells.

15 17. A method according to claim 16 wherein the virus comprises an echovirus serotype or a modified form thereof.

18. A method according to claim 16 wherein the virus is selected from a group consisting of EV1 and EV8.

19. A method according to claim 17 wherein the virus is a modified echovirus.

20 20. A method according to claim 19 wherein the virus has been modified to enhance ability of the virus to infect the abnormal cells.

21. A method according to claim 19 or 20 wherein the modified echovirus is a modified form of an echovirus selected from a group consisting of EV1 and EV8.

25 22. A method according to any one of claims 16 to 21 further comprising comparing ability of the virus to infect and cause death of the cells with a different virus subjected to steps (b) and (c) utilising another sample of the cells and which recognises $\alpha_2\beta_1$ for infectivity of the cells.

23. A method according to claim 22 wherein the different virus is a different echovirus or modified form thereof.

30 24. A method according to any one of claims 16 to 23 wherein the cells are cancer cells.

25. A method according to claim 24 wherein the cancer cells are cells of a cancer selected from a group consisting of ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer and colorectal cancer, or have spread from

ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer or colorectal cancer.

26. A method of screening a virus for ability to infect and cause death of abnormal cells from a mammal to evaluate administering the virus to the mammal for treatment of the abnormal cells, the method comprising:

- (a) selecting the virus;
- (b) treating a sample of the abnormal cells from the mammal with the virus for a period of time sufficient to allow infection of the cells by the virus; and
- (c) determining whether the virus has infected and caused death of at least some of the abnormal cells;

wherein the virus is selected from echoviruses and modified forms thereof, which recognise $\alpha_2\beta_1$ for infectivity of the abnormal cells.

27. A method according to claim 26 wherein the virus comprises an echovirus serotype or a modified form thereof.

28. A method according to claim 26 wherein the virus is selected from a group consisting of EV1 and EV8.

29. A method according to claim 27 wherein the virus is a modified echovirus.

30. A method according to claim 29 wherein the virus has been modified to enhance the ability of the virus to infect the abnormal cells.

31. A method according to claim 29 or 30 wherein the modified echovirus is a modified form of an echovirus selected from a group consisting of EV1 and EV8.

32. A method according to any one of claims 26 to 31 further comprising comparing ability of the virus to infect and cause death of the cells with a different virus subjected to steps (b) and (c) utilising another sample of the cells and which recognises $\alpha_2\beta_1$ for infectivity of the cells.

33. A method according to claim 32 wherein the different virus is a different echovirus or modified form thereof.

34. A method according to any one of claims 26 to 33 wherein the abnormal cells are cancer cells.

35. A method according to claim 34 wherein the cancer cells are cells of a cancer selected from a group consisting of ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer and colorectal cancer, or have spread from ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer or colorectal cancer.

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36. A method for inducing an immune response in a mammal against abnormal cells expressing $\alpha_2\beta_1$, the method comprising infecting abnormal cells in the mammal with virus selected from echoviruses, and modified forms and combinations thereof, whereby lysis of at least some of cells is caused.

5 37. A method according to claim 36 wherein the virus comprises an echovirus serotype of modified form thereof.

38. A method according to claim 37 wherein the virus is selected from the group consisting of EV1 and EV8.

39. A method according to claim 37 wherein the virus is a modified echovirus.

10 40. A method according to claim 39 wherein the virus has been modified to enhance ability of the virus to infect the abnormal cells.

41. A method according to claim 39 or 30 wherein the modified echovirus is a modified form of an echovirus selected from a group consisting of EV1 and EV8.

15 42. A method according to any one of claims 36 to 41 wherein the abnormal cells have up-regulated expression $\alpha_2\beta_1$.

43. A method according to any one of claims 36 to 42 wherein the virus is administered to the mammal in combination with a further virus which infects the abnormal cells.

20 44. A method according to claim 43 wherein the abnormal cells express ICAM-1 and the further virus recognises ICAM-1 for infectivity of the abnormal cells.

45. A method according to claim 44 wherein the further virus is a Coxsackievirus or modified form thereof.

46. A method according to claim 45 wherein the Coxsackievirus is a Coxsackievirus serotype selected from A13, A15, A18 and A21.

25 47. A method according to any one of claims 36 to 46 wherein the abnormal cells are cancer cells.

48. A method according to claim 47 wherein the cancer cells are cells of a cancer selected from a group consisting of ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer and colorectal cancer, or have spread from ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer or colorectal cancer.

49. A method according to any one of claims 36 to 48 wherein the virus is administered topically, systemically or intratumorally to the mammal.

35 50. A pharmaceutical composition for treating abnormal cells in a mammal, comprising an inoculant for generating virus to treat the cells such that at least some of

the cells are killed by the virus together with a pharmaceutically acceptable carrier, wherein the virus is selected from echoviruses, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$ for infectivity of the cells.

5 51. A pharmaceutical composition according to claim 50 wherein the virus comprises an echovirus serotype or modified form thereof.

52. A pharmaceutical composition according to claim 51 wherein the virus is selected from the group consisting of EV1 and EV8.

53. A pharmaceutical composition according to claim 49 wherein the virus is a modified echovirus.

10 54. A pharmaceutical composition according to claim 51 wherein the virus has been modified to enhance ability of the virus to infect the abnormal cells.

55. A pharmaceutical composition according to claim 53 or 54 wherein the modified echovirus is a modified form of an echovirus selected from a group consisting of EV1 and EV8.

15 56. A pharmaceutical composition according to any one of claims 50 to 55 wherein the abnormal cells are cancer cells.

57. A pharmaceutical composition according to any one of claims 50 to 56 wherein the pharmaceutical composition is for topical administration or injection.

20 58. An applicator for applying an inoculant to a mammal for generating virus to treat abnormal cells in the mammal, wherein the applicator comprises a region impregnated with the inoculant mammal such that the inoculant is in contact with the mammal, and the virus is selected from echoviruses, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$ for infectivity of the cells..

25 59. An applicator according to claim 58 wherein the region impregnated with the virus comprises padding or wadding for being held in contact with the mammal.

60. An applicator according to claim 58 or 59 wherein the abnormal cells are abnormal skin cells and the applicator further comprises one or more adhesive surfaces for adhering to skin of the mammal.

30 61. An applicator according to any one of claims 58 to 60 in the form of a patch or sticking plaster.

62. Use of an inoculant for generating virus in the manufacture of medicament for inducing an immune response against abnormal cells in a mammal, where the virus is selected from echovirus, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$, for infectivity of the abnormal cells.

63. Use of an inoculant for generating virus in the manufacture of medicament for inducing an immune response against abnormal cells in a mammal, where the virus is selected from echovirus, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$, for infectivity of the abnormal cells and kill the cells.